ON THE OCCURRENCE OF PLACENTATION IN THE SCINCID LIZARD *LYGOSOMA ENTRECASTEAUXI.*

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(Plates xlvii-xlix and three Text-figures.)

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Contents.

i. *Introductory.*

During the visit of a party from the University of Sydney to Barrington Tops in January and February of this year (1925) under the leadership of the senior author, Dr. I. M. Mackerras, who was examining the reptiles obtained for Haemoprotozoa, discovered that two of the common skinks present, *Lygosoma quoyi* Dum. et Bibr., and *L. entrecasteauxi* D. & B., were viviparous, and were carrying advanced young. In view of the possible interest of this discovery, a series of both species was collected.

The former of these species has already been recorded as viviparous by Lucas and Frost (1893), but these authors state that the latter species is oviparous in Victoria, laying from three to five eggs in January. This suggests a possible difference in reproductive habit on high and low ground, which, should it be confirmed, offers points of curious interest.

The reproductive phenomena of Australian reptiles have attracted small attention from workers in zoology, so that little precise information exists concerning oviparity and viviparity amongst the different groups. Lucas and Le Souef (1909) mention that most of the venomous snakes are viviparous, but quote only two lizards as being so, viz., *Tiliqua nigrolutea* and *Trachysaurus rugosus,* both belonging to the Scincidae. Lucas and Frost (1893) have, however, recorded four other skinks as viviparous. The remaining families of lizards occurring in Australia, viz., Geckonidae, Pygopodidae, Agamidae, and Varanidae are, as far as is at present known, all oviparous. Amongst the skinks, we now know the following species to be viviparous:—

Trachysaurus rugosus Gray	Rec. by Haacke (1885)
Tiliqua scincoides White	,, ,, do.
Tiliqua nigrolutea Gray	,, ,, Lucas & Le Souef (1909)

Egernia striolata Peters	Rec. by Lucas & Frost (1893)
Lygosoma (*Hinulia*) *quoyi* D. & B.	,, ,, do.
L. (*Siaphos*) *maccoyi* Lucas & Frost	,, ,, do.
L. (*Liolepisma*) *pretiosum* O'Shaugh.	,, ,, do.
and	
Egernia whitei Lacep.	Now recorded from speci-
Lygosoma (*Liolepisma*) *entrecasteauxi* D. & B.	mens in our collection.

It seems likely, when the large Scincid fauna of Australia is adequately examined, that a considerable number of species will prove to be viviparous, since this mode of reproduction offers obvious advantages.

Definite placentation has been recorded only in the case of *Tiliqua scincoides* by Flynn (1923); although Haacke (*l.c.*) mentions certain relations between the uterine wall and that of the yolk-sac in *Trachysaurus rugosus.* Of the nine species mentioned above as being viviparous we have examined pregnant females of five, the first, second, fifth, eighth, and ninth. In *L. entrecasteauxi* alone have we found a definite placenta comparable with that of the classic *Chalcides tridactylus* described by Giacomini (1891). The remaining four exhibit highly vascularized external allantoic and "uterine" walls, with their respective circulations in close apposition, but no marked placental area such as we describe below. The conditions would appear to be much the same as obtain amongst the species of *Chalcides* (Graham Kerr, 1919, p. 480), of which one only has a definite placenta, while in others there is merely close apposition between the maternal and foetal circulations. Since Flynn (1923) has recorded the existence of an allantoplacenta in *Tiliqua,* and has promised a further account of it, we do not propose to do more than draw attention to the fact that in a pregnant *T. scincoides* in our collection, containing young 140 mm. in length, we find neither the villous folding of the uterine wall, nor the great modification of the chorionic epithelium which occurs in the European *Chalcides tridactylus*, and in *Lygosoma entrecasteauxi* as described below.

We have to thank Mr. J. R. Kinghorn, C.M.Z.S., of the Australian Museum, Sydney, for identification of our lizards; Mr. G. Burfield, of the Department of Physiology, University of Sydney, for making the photomicrographs reproduced in Plate xlviii; and Mr. Horace Weekes for the drawings which appear on Plate xlvii.

ii. *Material and Methods.*

The material at our disposal comprises nine pregnant females of *Lygosoma entrecasteauxi* collected at Barrington Tops, 150 miles north of Sydney, at a height of 4,500 to 5,000 feet, during the last fortnight of January and the first week of February, 1925, by various members of the Sydney University Party. The lizards were fixed and preserved entire, after opening up the ventral body-wall to expose the contained foetuses within the uteri, in a fluid recommended by Bles (1905) which has proved to be excellent for general field work, since animals may be preserved in it indefinitely. It has given a good general fixation, which is not, however, in the case of all kinds of cells, good enough for fine histological work. It has, moreover, one serious fault in that it renders tissues very brittle, and so makes difficult subsequent dissection of material fixed in it.

The embryos examined are all approximately in the same stage of development, 30 to 40 mm. in length, indicating a fairly long gestation period, since comparatively small differences are shown amongst those collected at random over a period of three weeks. The breeding season would appear, moreover, to be a very regular one, as numerous males were vainly sacrificed in an endeavour to secure younger stages, there being no external differences between the sexes. All the obvious females showed by their bodily contours an advanced condition of pregnancy.

The present communication, then, describes what is practically a single stage, and that much too advanced for the satisfactory elucidation of the earlier ontogenetic features, which should prove of considerable theoretical interest. The authors will endeavour to obtain a series of earlier stages during the coming summer, but have thought it advisable, in view of the interest attaching to the discovery of a second fairly complex allantoplacenta in the Scincidae, to publish the description which follows of the actual placental condition, hoping to be able on a future occasion to give some account of its development.

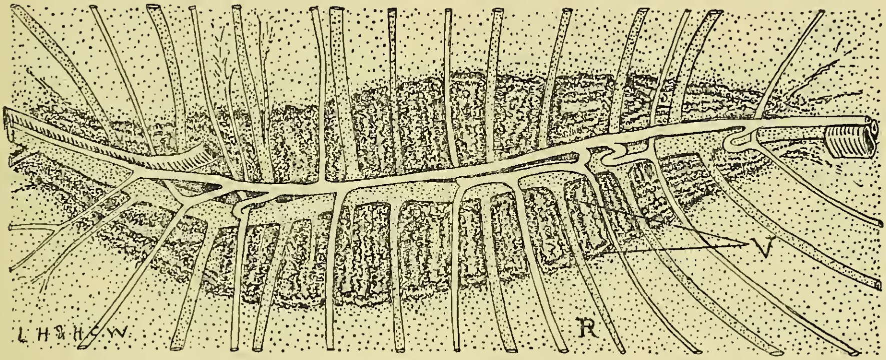
Examination was made by means of whole mounts of both foetal and maternal placentae, and of the two together; and by means of paraffin sections of the conjoint placental area alone, and of the whole uterine sac with its contained embryo, taken transversely to the long axis of both oviduct and foetus. All preparations were stained with Ehrlich's haematoxylin, eosin being used as a counterstain for the sections.

iii. *Anatomical Relations.*

Of the nine pregnant females available, two contained seven young, five others five each, and the remaining two only three. It seems remarkable that the number should be odd in every one of nine examples, and we cannot find any explanation for this condition. It is obvious, when large young are produced in a small parent, that these must be somewhat finely adjusted to the space available for their development. One would therefore expect the row of embryos contained in one oviduct to alternate with those contained in the other, and this is found to be so. Moreover, those lizards which contain three young are the smallest of our specimens, but size does not appear to offer any explanation in regard to the five individuals which have five contained young, since, if size were a factor, there is sufficient variation amongst them to lead one to expect four young in one case and six in another.

The number of embryos in either oviduct seems a matter of chance. From three of our specimens the embryos had been removed for dissection and sectioning without a note having been taken of their positions. Of the remaining six, four had more embryos in the right oviduct than in the left. The contained foetuses in our specimens are all well advanced, and approaching full term. We have selected for examination what appears to be the youngest stage present, in which we find the foetus to be 26 mm. in total length when extended; as well as several older stages, in which the average total length is 33 mm. There is, therefore, not much difference between our youngest and oldest stages. The embryos within their membranes cause the oviducts to exhibit a series of moniliform swellings (Pl. xlvii, fig. 1) very closely adpressed end to end, so that they adjoin by flat surfaces, and are connected one to another by a short, strap-like section of the oviduct which joins the centres of these apposed surfaces. These swellings are called by Giacomini for *Chalcides* "incubatory chambers", but we use the term "uterus" for the whole uterine portion of the oviduct, which must have become greatly distended from its original condition, since the small ovaries lie on its mesial border at the middle of its length, and not anteriorly. Mutual pressure of the embryos contained in the two uteri brings about a flattening of their mesial faces also. Each swelling is thus roughly oblong in surface view, with an average length of 6 mm., by 9 mm. wide.

Certain structures are more or less visible through the thin and much distended uterine wall. As the parent lies upon her back, with the ventral body-wall opened up, and the uteri exposed, the region of the yolk-sac for each embryo shows as a creamy area, ventral and lateral, i.e., anti-mesometrial in position. On turning back the uterus of either side, the allantoplacental region is seen to be dorsal and mesial, and the maternal placental area shows as an opaque patch (Pl. xlvii, fig. 1) plainly visible to the unaided eye.



Text-fig. 1.—Placental area of uterine wall viewed as a transparent object. Arteries white, veins stippled; R, area shown in Text-fig. 2*a*; V, villous ridges; × *circ.* 12 diameters.

The series of prominent blood vessels, arteries and veins parallel and, in general, alternate, which runs out from this area round the uterine wall (Pl. xlvii and xlviii, fig. 1; Text-fig. 1) stops at the margin of the yolk-sac, and so helps to delimit the boundaries of the latter. The foetus lies with its long axis parallel to that of the parent, and with the yolk-sac upon its left, and the main portion of the allantoic cavity upon its right side. It is thus in an obliquely vertical position, lying upon its back, with its head curled up towards the dorsal side of the parent body, and directed anteriorly when in the left uterus, posteriorly when in the right. The tail is folded forwards over the ventral surface of the foetal body as far as the head, close to which it turns inwards and ends in a close spiral. The same general relations hold for all our specimens with the exception of one in which the yolk-sac area is distinctly more mesial in position.

On dissecting away the uterine wall, and disclosing the foetus lying within its membranes, it is at once obvious that the allantoic sac does not completely surround the whole periphery, but turns inwards round the edge of the yolk-sac and passes internal to the latter structure. Externally, the yolk-sac is covered only by the serosa. It is, at the stage we discuss, apparently much reduced, and extends over only about one-fourth of the circumference of the "blastocyst" in section, the remaining three-fourths being occupied by the allantois. The yolk-sac is mushroom-shaped, the stalk ascending to meet the allantoic stalk in a common umbilical stalk close to the mid-line of the foetal body at a little distance in front of its hind limbs. The relations may be seen at a glance by reference to the stereogram reproduced as Figure 3 of Plate xlvii. By reference to the same figure, the allantoic stalk is seen to pass directly upwards and to open out, above the embryo, into a wide allantoic cavity which passes round the foetus in all directions to extend, at the lower pole, inwards between it and the yolk-sac, ending, in a ring-like manner, around, but quite free from, the yolk-stalk at its junction with the sac.

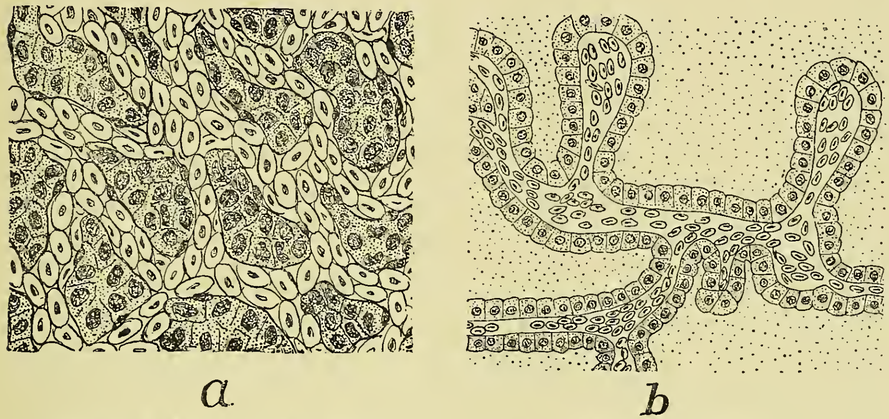
To the amnion we have not given any particular attention. It is an extremely delicate membrane, which arises from round the base of the umbilical stalk, and closely invests the foetus through all its curvatures. We can find no trace, at this stage, of a sero-amniotic connection.

There is no trace of egg-shell or of secondary membranes apparent in our stages, nor can we determine the origin of a coagulum which lies between the uterine wall and the serous membrane in the yolk-sac region (Pl. xlviii, fig. 4; Pl. xlix, fig. 3), which may be the remains of albumen, or may be a uterine secretion.

iv. *The Placentae.*

Lygosoma entrecasteauxi possesses both an omphaloplacenta and an allantoplacenta, the latter being much more highly organized in the stages at our disposal. The arrangement of the blood-vessels in the uterine wall (Pl. xlviii, fig. 1, and Text-fig. 1) suggests that possibly the yolk-sac placenta was more important at an earlier stage. These vessels are so very prominent, and end so suddenly at the periphery of the area of uterine wall overlying the yolk-sac, entering into small relations with the sparse vessels of this region, that the arrangement suggests a change-over from an earlier and more richly vascular omphaloplacenta to a later allantoplacenta. We find in *Lygosoma quoyi* that the maternal portion of the omphaloplacenta is very richly vascular. In this form, as far as our observations have gone, there is no definite allantoplacenta, and the yolk-sac placenta is very prominent in stages seemingly equivalent to those of *L. entrecasteauxi* which we have examined, so that the two are not strictly comparable. In *Chalcides*, according to Giacomini (1891, p. 356), the yolk-sac placenta "se developpe tardivement et reste rudimentaire".

(*a*) *Maternal blood-circulation.*—We reserve a full statement of the details of maternal circulation for a future occasion, when we shall have earlier developmental stages for examination and comparison. Here we give merely a brief outline of the arrangement of vessels in the uterine wall. A large artery and vein run longitudinally on the dorsal side of the uterus, slung in a fold of the mesometrium, and standing out as a prominent ridge bisecting the allantoplacental areas (Pl. xlvii, fig. 1, *Ut. b. v.*). The vein receives a single large branch vein from each placental area, to which latter the artery sends also a single branch. The placental artery runs dorsal to the vein, and branches in a manner best seen by reference to Text-fig. 1, which shows a series of paired branches running parallel with one another transversely round the uterus. The placental vein is branched in a somewhat similar fashion, the branch veins in general alternating with the branch arteries. Arteries and veins extend to the periphery of the yolk-sac area, as mentioned above. The arteries give off frequent minute branches to the capillary network of the uterine wall, which is extraordinarily rich, and ramifies as a close plexus over the whole of its area (Text-fig. 2, *a*). From this reticulum small vessels enter the branch veins, and are carried back to the main placental vein. Over the yolk-sac area there remain only a few small irregularly ramifying vessels. The maternal portion of the allantoplacenta appears as a fusiform to elliptical opaque whitish area (Pl. xlvii, fig. 1, *Plac.*), the opacity being due to the fact that the internal face is raised up into a series of villous ridges, covered by a cubical epithelium, and containing otherwise only capillary blood-vessels with their endothelial lining. At either end of the placenta these ridges run in a general longitudinal direction, but in the middle region, for about half its length, they are arranged transversely. Each apparent major ridge is formed by the



Text-fig. 2.—(*a*) Vascular reticulum in uterine wall in optical section; (*b*) Uterine villous fold in optical section.

closely sinuous plaiting of a much narrower fold, the whole having a very complex form. The vessels occupying these ridges are fed by short branches from the main branch arteries and veins. A prominent ridge runs round the periphery of the whole area. The nature of these ridges is shown in Text-figure 2, *b*; and some idea of their complexity may be gained by reference to the section reproduced as Figure 3 of Plate xlviii.

(*b*) *Foetal blood-circulation.*—A section of the umbilical stalk is shown in Plate xlix, figure 6, from which it will be seen that the endodermal lining surrounds a simple cavity, formed by the junction of the yolk-sac and the allantoic stalks, which passes into the body of the foetus, and runs posteriorly for some considerable distance before entering the gut. The splanchnopleural mesoderm is thrown into somewhat intricate folds, wrapping round the various blood-vessels. Close to the body, the dorsal space surrounded by these folds is filled with proliferated mesenchyme, surrounding the umbilical and vitelline veins, which are both single, and one of the paired vitelline arteries. An umbilical artery, formed by the junction of two distinct arteries in the allantoic stalk (see Pl. xlix, fig. 5), and the other vitelline artery lie on the ventral side of the stalk lumen. When these vessels are traced into the body of the embryo, the umbilical vein enters the liver, and there joins with the intra-hepatic vessels, and with the ductus venosus; the umbilical artery is found to branch from the aorta posteriorly, and not from the sciatic artery as in the chick; the vitelline vein enters the liver; and the two vitelline arteries are given off separately from the aorta. As the yolk-stalk is followed outwards, it is seen that a second and smaller vitelline vein enters the umbilical vein.

As the allantoic stalk passes upwards, the umbilical artery divides into two branches, which come to lie on either side of the umbilical vein. When the allantoic stalk opens out into the sac, the blood vessels are carried across the lumen of the latter in a remarkable and interesting manner direct to the placental region. In three of the four embryos of which we have serial sections, a pleated fold arises from the inner allantoic wall (Pl. xlvii, fig. 3; xlviii, fig. 2; xlix, fig. 5, *Pl.*), within the inner free edge of which the vessels are carried. Since this pleat is covered externally with endoderm continuous with that lining the allantoic cavity, there would appear to be no doubt that it has arisen originally as a fold of the allantoic wall. The base of this fold is of considerable extent, and can be traced round the inner wall of the allantois almost to the yolk-sac region. Figure 5 of Plate xlix shows clearly enough the way in which the vessels link up; and in the photomicrograph which appears as Figure 2 of Plate xlviii the umbilical vein is shown actually in transition to the placental region. In our fourth series of sections a somewhat different condition occurs. In place of the flat fold, a blunt finger-like process is pushed out from the inner wall, and passes across the lumen to bring about the same ultimate result. Since the fold occurred in three embryos which were dissected (and puzzled us considerably until sections disclosed its actual nature) as well as in three sectioned embryos, it would appear to form the normal method of transference.

Flynn (1923, p. 77, and Fig. 1, p. 74) describes and figures for *Tiliqua scincoides* a somewhat similar arrangement. He writes:—"But the outer wall of the vesicle is also supplied by vessels which leave the allantoic stalk near the body of the embryo, and pass right across the vesicle to ramify through the mesenchymal layer of the placental face of the allantois. The presence of such a method of transmission of allantoic vessels—by direct cellular bridges across the allantoic cavity—is interesting in that it has already been recorded by Hubrecht for a monodelphian mammal, *viz.*, *Erinaceus*". Flynn figures the allantoic stalk as passing down posterior to and parallel with the yolk-stalk, but his "cellular bridge" offers so close an analogy with the allantoic stalk of *Lygosoma entrecasteauxi*, and with its continuation as the edge of a pleated fold, that we suspect further examination will prove the relations to be the same in both forms. In any event, the condition shown by *Lygosoma* has nothing in common with that described by Hubrecht for the hedgehog.

(*c*) *The omphaloplacenta.*—This structure is obviously in a condition of regression, and it would serve no purpose to discuss in any detail the scarcely different stages which we possess. In Figures 4 and 5 of Plate xlviii, the general relations of our extreme stages are shown. The yolk-sac is more voluminous in the latter than in the former, and contains a homogeneous coagulum within portion of its cavity, which resembles generally that found lying between the serosa and the uterine wall in the former, which is shown in more detail in Figure 3 of Plate xlix. Since this coagulum stains deeply with haematoxylin, it differs altogether from that found in the allantoplacental region, which remains practically colourless. Possibly this coagulum represents the remains of an albuminous coat, but it seems more likely that it is some trophic secretion of the uterine wall, which has gravitated to the bottom of the cavity after fixation.

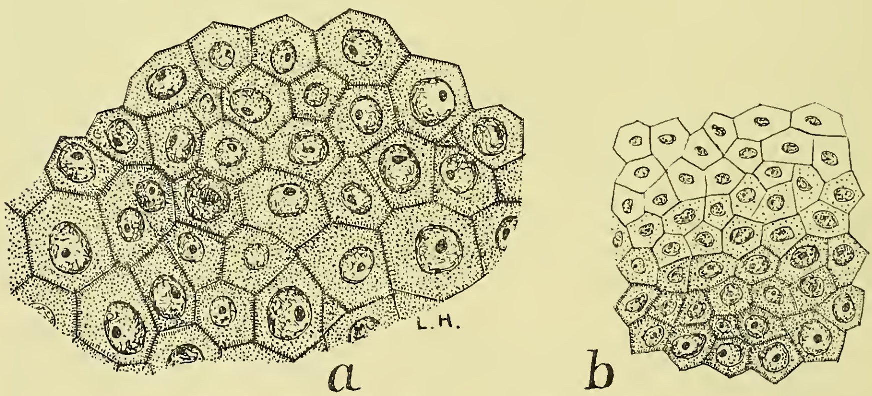
The uterine epithelium is here cubical, the cells measuring on an average .028 mm. in height by .016 to .022 mm. in width, which is approximately the same size as those covering the villous ridges of the allantoplacenta, and greatly larger than those of the intermediate area. The epithelium is underlain by a network of small capillaries, which here and there appear to cause slight bulges of the overlying cells, but no folding into ridges can be seen.

The chorion consists of an ectodermal layer of cubical cells measuring .03 mm. in height and width, bounded internally by a narrow layer of mesoderm. The endodermal cells of the yolk-sac itself are relatively enormous, measuring .124 mm. in height by .05 mm. in breadth. Their general form is indicated in Figure 4 of Plate xlix. They show large, densely-staining nuclei, and the cytoplasm is extensively vacuolated, and is packed to a varying extent with large yolk sphaerules.

(*d*) *The allantoplacenta.*—The general disposition of the maternal portion of this organ has already been dealt with in the description above of the maternal circulation. The thickened and plaited area which is clearly delimited by a ridge running round its margin, measures 10 mm. long by a little more than 3 mm. wide in the example figured (Text-fig. 1; Pl. xlviii, fig. 1). The thickened chorionic epithelium which characterizes the foetal portion of the placenta is of slightly greater extent, its margin passing that of the maternal area right round the periphery. The way in which the inner face of the uterine wall is folded leaves a series of crypts lying between the complicated villous ridges. There is, however, no interpenetration of chorionic ectoderm into these crypts, maternal and foetal layers being closely apposed, so that the general form of the villous ridges impresses itself upon the chorionic surface, but without any kind of fusion whatever. However carefully the placental area is dissected away, the maternal and foetal membranes at once become free from one another, and a certain degree of separation follows upon careful embedding and sectioning of the whole blastocyst within its uterine chamber. Reference to Figure 2, Plate xlviii will show this separation, as well as the fact that the sectional outline of the maternal villi is complementary to that of the foetal chorionic ectoderm. Drawings made by means of the camera lucida, cut out in profile and then apposed, have shown that the two membranes fit closely together. Flynn (1923, p. 76) describes the connection between these epithelia in *Tiliqua* as very intimate, and suggests a certain degree of plasmodium formation, and of interpenetration of foetal cells between those of the uterine wall. Our photographs and drawings show conclusively that such things do not occur in *Lygosoma*. We have to do simply with two epithelial sheets with every cell clearly bounded, which are in a position of close apposition, but are in no way joined or fused, nor do they exhibit amoeboid processes or plasmodium formation. Certain portions of the photomicrograph which forms Figure 3 of Plate xlviii might perhaps be interpreted as suggesting this last, but examination of the actual sections shows that these represent sections of villi cut tangentially.

The maternal crypts thus form chinks or interstices between the two membranes, and are for the most part filled with a ropy, non-staining coagulum which, as already mentioned, differs from those found in the yolk-sac region. The villous ridges project on an average .1 mm. from the uterine wall. The cells of the epithelium covering them measure in section .025 mm. by .017 mm. At the edges of the placental area there is no transition of this thickened epithelium into the squamous type which lines the uterus, as occurs in the foetal ectoderm. The epithelium is strongly ciliated, and the last lateral thickened and ciliated cells of either side of any section adjoin a non-ciliated squamous cell of the general surface. The cells have a densely granular, dark-staining cytoplasm, but show no signs of vacuolization, or of glandular activity. No specialized glands occur over the area.

The cells of the chorionic ectoderm are columnar and extremely elongated, being in all cases twice as long as broad, while cells occur quite frequently which are three times as long as broad. The free surfaces are mammillate in form with a dark border in section and strongly ciliated. The whole of the cytoplasm is densely granular and shows little sign of vacuolization. The nuclei are large and vesicular, in many cases occupying almost the whole width of the cell; most of them show one large densely staining nucleolus, central or slightly eccentric in position, but in some there are two nucleoli placed at opposite poles. No signs of active multiplication are apparent in the form of mitotic figures, but this is possibly due to the slowness of fixation since some cells show two small nuclei closely apposed to one another along the long axis of the cell while here and there two extremely narrow cells with their nuclei side by side suggest recent division, in which the cell has not yet grown to its maximum size. The outline of the free surface of this epithelium in section is very irregular and consists of a succession of bays and prominences which correspond to the prominences formed by the uterine villous ridges of the maternal placenta. The extent of thickened ectoderm slightly exceeds that of the maternal placental area, but peripherally there is a marked flattening out of the epithelium until at the margin of the foetal placenta the cells are absolutely cubical and only about one-quarter the height of those of the middle of the placental region. These cubical cells have densely staining nuclei right at their bases and exhibit a very definite border of denser material which stands out clearly from the remainder of the cytoplasm. Passing beyond the margin of the placental region the chorionic ectoderm becomes squamous, and the cytoplasm loses its granular appearance and the nuclei their vesicular form, the latter becoming ellipsoid in shape and staining very densely and uniformly (Text-fig. 3, *b*).



Text-fig. 3.—Chorionic ectoderm in surface view; (*a*) in middle of foetal placenta; (*b*) transition zone at edge of same.

The somatopleural mesoderm has throughout most of the placental region a fairly complex structure. The main vascular network lies close at the base of the chorionic cells, so much so that in transverse section the extremely numerous capillary vessels have the appearance of piercing the cell bases. These minute vessels appear in all cases to have endothelial lining, the lumen being surrounded by an extremely fine but quite definite membrane, which is frequently enlarged to surround sickle-shaped nuclei which are curved round the narrow lumen of the vessel. Internal to these capillaries lies a definite cellular membrane with flattened nuclei lying parallel to the bases of the ectodermal cells. Internal to this is a sheet of mesenchymatous connective tissue, thin over the greater part of the placental region but forming a fairly thick cushion from the caudal end, commencing where the allantoic vessels crossing the cavity attach themselves to the placental region, and running round in the direction of the yolk-sac. This tissue consists of a loose reticulum containing scattered nuclei, and is bounded internally, by a second endothelial layer. Through this connective tissue, throughout most of its extent, there runs a thin sheet of muscle fibres.

v. *Comparison with* Chalcides.

The literature of placentation in *Chalcides* is not very voluminous, and is, apparently, confined to a period of about five years following upon Giacomini's original announcement in 1891. We quote below (p. 485) a number of titles having reference to it, but most of these papers, unfortunately, do not appear to be available in Australia, and we have had access to two only of Giacomini's papers, viz. what is apparently a reprint of his original account in the *Monitore zoologico italiano* which appears in the *Archives Italiennes de Biologie*, Vol. xvi, for 1891; and a brief summary of his results in the *Anatomischer Anzeiger* for the same year. Lesser references to his results occur in Strahl (1891), Kerr (1919), and Schauinsland (1906).

In *Chalcides*, apparently in the vicinity of Sienna, although this is not definitely stated, ripe ova from 2.5 to 3 mm. in diameter descend into the oviduct about the middle of May, and are there fertilized. Neither an albuminous layer nor secondary membranes are formed about them. They differ from those of oviparous reptiles in the small yolk content. The young are born, free from foetal envelopes, at a length of 95 mm., after a gestation period of three months. The number of ova varies between 5 and 15, the average being 8 to 10, but all do not necessarily develop. The ova become spaced out in the oviducts, producing ultimately moniliform swellings which persist for some time after birth. The ova lie with their animal pole mesometrial in position. The embryo lies transversely to the long axis of the blastocyst, upon its left side on the yolk-sac, with the allantois, when first developed, upon its right side. The latter grows down ventrally, but never passes round the yolk-sac externally, that is to say, between it and the serosa. The placental area is elliptical, the villous ridges of the uterine wall running in the general direction of the long axis of the ellipse. The chorio-allantoic membrane shows ridges corresponding to the "crypts" in the uterine wall, but there is no close interlocking between the two membranes, which are easily separable, and between which interstices filled with coagulum occur. The coagulum is stated to be the product of the thickened epithelium covering the uterine villi, and may be in part produced by the cytolysis of proliferated cells, but this is not certain. The blood circulation is not described in detail, but it is mentioned that the vascular network of the foetal is richer than that of the maternal placenta, so much so that in places on the peripheral region the meshes surround only one or two epithelial cells.

An omphaloplacenta precedes the allantoplacenta, but is not very highly developed, and is marked chiefly by the persistence of thickened chorionic ectoderm in this region. The chorionic ectoderm of the allantoplacental region is uniformly and strongly thickened, much more so than in the yolk-sac region, the cells being narrow and very high, with granular contents. This epithelium is thrown into villosities, which correspond to the "crypts" in the uterine wall, and the bases of these villi are underlain by a rich vascular network, as are those of the uterus.

These last arise before those of the chorion and are, in general, narrower in form. They are occupied internally by a delicate reticular connective tissue, which supports the capillary vessels, and are covered by a simple epithelium of a secretory character, which is much thicker than that lining the non-placental portion of the wall. The cell contents are dark and granular at their bases, becoming light and transparent towards their free faces.

Outside the area of placental modifications, both uterine wall and chorio-allantoic membranes show closely apposed and extremely rich capillary networks. No special description is given of the method by which the allantoic vessels reach the placenta, but from words used (1891, p. 344):—". . . l'allantoïde, en s'étendant dans le cœloma blastodermique ou externe, sous forme d'une vésicule revêtue, intérieurement, par l'entoblaste, et extérieurement par la lame viscérale du mésoblaste (feuillet connectif ou vasculaire de l'allantoïde) dans laquelle courent les vaisseaux allantoïdiens, ne se soit pas encore adossée, par sa face externe ou connective à la face interne de la vésicule séreuse, cependant, cette dernière commence déjà à se pourvoir etc.", it would seem that the vessels pass round the allantoic wall, and are not carried across the cavity by any special contrivance.

We are handicapped in making a comparison between *Chalcides* and *Lygosoma* by the fact that we have not access to the complete descriptions of Giacomini. The figures given in the paper on which we have chiefly to rely illustrate only the external features of the blastocyst. We do not even know whether the Italian embryologist has published figures illustrating the histological relations in *Chalcides*, but the fact that no such figures have been reproduced in any general work known to us seems to imply that none exists. Moreover, we have but one stage, and not a developmental series, for comparison.

Despite these limitations, however, it is possible to make some comparison, and to indicate that the correspondence between the two forms is, in general, remarkably close. *Chalcides* is a larger lizard, producing a greater number of young at a birth. The general relations of the embryo to its membranes are identical, as well as the relations of the foetus to the maternal organs. Both have an omphaloplacenta of the same type, which in *Chalcides* is at no stage very much developed, but in *Lygosoma* seems to indicate a more actively functional condition at an earlier ontogenetic stage. In both, the allantois does not surround the yolk-sac externally; the epithelial lining of the uterine wall changes in character and becomes notably thickened over two placental areas at opposite poles; and the chorionic ectoderm is modified in an apparently identical manner at the same places. In both, the mesodermal elements of the chorion and allantois fuse to form a complex allantochorion, which carries a rich vascular network in correspondence with a similar plexus in the uterine wall, the two being closely apposed. In the allantoplacental region each form shows a series of interstices between foetal and maternal tissues, filled with a coagulum identical in character. Finally the foetal and maternal membranes are easily separable in both, and there is no actual fusion between them.

Lygosoma entrecasteauxi differs from *Chalcides tridactylus* in the following points:—

1. The allantochorion is not thrown into villous folds corresponding to those of the uterine wall.

2. The villous ridges of the latter run chiefly in a transverse direction and not longitudinally.

3. Both uterine and chorionic epithelia are ciliated.

4. The allantoic vessels are carried across the cavity in a special fold of the inner allantoic wall, and do not pass round the periphery of the allantoic sac.

vi. *Theoretical Considerations.*

It is not proposed at this stage to enter upon a detailed discussion of the significance of the reptilian allantoplacenta, but certain outstanding features appear to demand brief comment. The parallelism between the occurrence of a fairly highly-organized placenta in two not very closely related Scincid genera, *Chalcides* and *Lygosoma*, accompanied in each case by indications of simpler placental conditions in related species, is remarkable. Brief references to the placental condition in *Chalcides* are fairly general in text-books, but Graham Kerr (1919) is the only embryologist known to us who has given expression to the importance of this phenomenon, and even he has not discussed its theoretical bearings. In the important discussions of placentation associated with the names of Van Beneden, Hubrecht, Assheton, Jenkinson, and Hill, no consideration has, so far as we are aware, ever been given to the possible value of a reptilian placenta in elucidating the evolutionary history of that organ. Giacomini's original papers are not available to us, but, so far as we are able to judge from the summaries and extracts from which we quote, he himself was fully seized with the importance of his discovery, originally announced much earlier by Studiati, and it is remarkable that his extensive work should have attracted so little attention.

Placentation in Scincid lizards, a specialized family of a specialized group of reptiles, obviously can have no direct genetic relationship with the analagous process in mammals. To our thinking, however, this very fact makes the reptilian placenta so much the more important, for there is here displayed an independent placentation in an otherwise non-placental group, which in many respects affords a very close parallel to mammalian conditions. If the statement of Lucas and Frost quoted above (p. 470) be confirmed, namely that *Lygosoma entrecasteauxi* is oviparous through part of its range, we should have the extraordinarily interesting condition of a species exhibiting in part normal sauropsidan development, and in part a placental mode; but it is probable that this statement rests upon a wrong conception of the contents of the oviducts, such as the same authors have put forward in the case of *Tiliqua scincoides*. Be that as it may, the conclusion seems to us inevitable that placentation is a functional adaptation which, given certain prerequisites, may have arisen independently on many occasions before the higher mammals settled down to the placental mode. These prerequisites comprise (1) an ectodermal layer which has entered into some metabolic relation with the external environment; (2) a yolk-sac; and (3) an allantois.

Without committing ourselves in any way to the view advanced by Hubrecht that the external layer of ectoderm of the anamniote larva is homologous with the mammalian trophoblast, we may justifiably commence an argument from function with this layer. The three prime functions to be provided for in the development of an embryo are respiration, nutrition, and excretion. In the anamniote larva, nutrition is provided for by means of the contained yolk-mass; excretion by the early establishment of archinephric ducts opening into a cloaca; so that the one function remaining to the external cellular layer is that of respiration. Since we hold the conventional view that the extra-embryonic phenomena exhibited by the amniota are the direct consequence of adaptation to a terrestrial environment, and to the evolution of a shelled egg, we view the extra-embryonic ectoderm primarily as a pneumatoblast, the yolk-sac remaining homologous with the internal yolk-mass of the anamniote larva, and the allantois being a new structure for the reception of poisonous excretory waste. The view we would take of amnion formation was developed before we had studied Hill's work upon the early ontogenetic features of marsupials, but appears to be supported by his observations, which have been confirmed by Hartmann, that there is an early division into formative and non-formative ectoderm, clearly differentiated morphologically, in the marsupial blastocyst. We would suggest that the formative ectoderm, possibly through the assumption of other functions, has lost its primarily pneumatoblastic character, and that it is due to the necessity for a maximum area of respiratory ectoderm that there is rapid proliferation not only in the direction of the yolk-sac, but also, in the form of amniotic folds, above the developing embryo. The extremely rapid proliferation noted by Wilson and Hill in *Ornithorhynchus*, in which the new-laid egg contains already a well-formed embryo, compared with the condition in birds, where the proliferation comes at a much later stage, since the egg at laying is only in an early stage of segmentation, and moreover displays a condition of arrested development, seems to us highly significant in this regard. For us, then, amniotic folds are primarily due to the necessity for a maximum extension of pneumatoblast, and the water-jacket function is secondary. This view, if acceptable, would cut the ground from under Hubrecht's argument that, if the prime purpose of the amniotic cavity be to subserve the function of a water-jacket, it should exist from the outset in a functionally perfect condition. Selenka's view of the mechanical origin of the amniotic folds owing to the downgrowth of the embryo into the yolk-sac is untenable on several grounds, such as the nonoccurrence of these folds in the Elasmobranchs, the absence from them of yolk, the necessity for accounting for proliferation of the edges of the folds, and the backward production of the reptilian amnion far beyond the limits of the embryo. The hypothesis outlined here would have the effect of dividing the nonformative ectoderm into two categories, pneumatoblast and ectodermal lining of the amniotic cavity. This last would, by virtue of its position, lose most, if not all, of its functional significance, and might tend towards elimination in the course of evolution. It seems to us that this suggestion may have a bearing upon the problem of amnion formation amongst the Mammalia.

The yolk-sac we look upon as homologous with the yolk-mass of the anamniote larva, increased in size to permit of the elimination of a larval period, and hence, owing to the mechanical difficulties involved in its enclosure, folded off from the body of the embryo. Since yolk has to be carried to all parts of the body of the embryo, and since blood is the carrier, blood is first formed in the yolk-mass of the anamniote, and in the splanchnopleural mesoderm covering the yolk-sac of the amniote. That is to say, a blood circulation comes into existence upon the yolk-sac to subserve the function of nutrition. Having come into existence, it is available for other purposes. An omphaloplacenta becomes possible by virtue of the existence of a trophic blood-circulation upon the surface of a yolk-sac. Similarly an allantois has come into existence for the reception of excretory waste, and its continually increasing bulk in the sauropsid egg, together with the gradual diminution of the yolk-sac as its contents are absorbed, brings a blood-circulation primarily concerned with the metabolism of its own cellular walls into relation with the external covering of the blastocyst, using the word in its widest sense, and makes possible an allantoplacenta. These views have been taught, we should suppose, in most schools of zoology for more than a generation, and their reiteration here would be gratuitous were it not for the paradoxical standpoint assumed by so distinguished an embryologist as Hubrecht, obsessed apparently by his preponderant studies of ontogenetic phenomena in the Mammalia.

It may seem presumptuous for workers with small experience in embryology to enter the lists against admitted champions, but we feel strongly that a consideration from the functional rather than from the morphological viewpoint must lead to a clearer understanding of the facts of early ontogeny; and we are of opinion that the independent occurrence of an allantoplacenta in two not very closely related reptiles justifies our functional point of view, and delimits the extent to which the existence of an allantoplacenta may justifiably be used as a phylogenetic argument. Thus the view of Hill (1897), still held by Flynn (1922), that the marsupials must be derived from placental ancestors, rendered inherently improbable by the persistence of the egg-shell in marsupials, becomes, to our thinking, untenable in the light of the fact that two reptiles have developed an allantoplacenta.

We agree with the general statement of Giacomini, who writes (1891, p. 354):—"Le placenta du *Seps* (*Chalcides*) confirme la loi générale établie par Ercolani, Turner, Romiti, et appuyée par Tafani, sur le mode de nutrition des embryons et des fœtus dans l'utérus, puisque les aptitudes à sécréter, constatées dans les élements de la portion maternelle, manifestent que celle-ci, dans certaines, périodes du développement, acquiert la fonction d'élaborer et de fournir un supplément de matériaux nutritifs au nouvel être. C'est pourquoi, ce n'est pas seulement pour les fonctions de la respiration que l'allantoïde, chez le *Seps*, prend, dans une région donnée, après s'être soudée avec la membrane séreuse de von Baer, la forme de placenta allantoïdien, mais bien, spécialement, pour les fonctions de nutrition, ou mieux encore, d'absorption des substances assimilables dont l'œuf fécondé, qui se développe, est dépourvu. Ici également, disais-je, nous trouvons l'unité du processus physiologique qui préside à la nutrition de tous les œufs en voie de développement et qui, même, sert à nous expliquer le pourquoi des dispositions particulières rencontrées dans les annexes fœtales du *Seps*".

We suggest, then, that there are three stages in placentation:—

(i) A *chorioplacenta*, primarily conditioned by the chorionic ectoderm (non-formative ectoderm, trophoblast), in accordance with the view of Minot. This we consider to be first respiratory, though later it may (and does) assume other functions. We have advanced a theoretical argument for its existence. In fact, thickening of the chorionic ectoderm over the embryonic region is well shown in sections of a 4 mm. embryo of *Tiliqua scincoides* in the collection of the Department of Zoology (Fig. 7, Pl. xlix) indicating a very early functional activity for this layer. We do not discriminate between a unilaminar and a bilaminar blastocyst wall, nor concern ourselves with the origin of the underlying lamina where this wall has a double character. The ectoderm is the important thing.

Flynn (1922, p. 541) has suggested an initial stage of placentation by means of the non-vascular wall of the bilaminar omphalopleure, to which he has applied the term "metrioplacenta". The term seems meaningless, and we cannot see that any particular virtue attaches to this region which is not shared by the ectoderm of the remaining portion of the blastocyst wall.

The chorioplacental stage is never omitted, and the use of the word "placenta" in compounding the term is meant to imply merely that the membrane is concerned in metabolic exchange between the surrounding environment and the embryo. Such use may not be considered happy, since the term is obviously capable of application to the sauropsidan egg, in connection with which the word "placenta" seems out of place. But it falls into line with the accepted terms which follow, and is an expression of what we believe to be a real homology, namely that of the sauropsidan chorionic ectoderm with the mammalian trophoblast.

(ii) An *omphaloplacenta*, which both ontogenetically and phylogenetically precedes the third stage, but which may be omitted in ontogeny.

(iii) An *allantoplacenta*, the final stage phylogenetically, which may never be attained (some marsupials), and which may follow directly upon the chorioplacental condition (most Eutheria), the omphaloplacenta having been eliminated.

Since it is our intention upon a future occasion to expand the thesis briefly outlined in this section, we have not burdened our list of references with the voluminous literature of placentation. References to the workers whom we mention will be found in the bibliographies to Hill's papers, which we include.

vii. *Summary and Conclusions.*

The present communication consists of a description of the placentation, at one stage of the Scincid lizard *Lygosoma* (*Liolepisma*) *entrecasteauxi*, with what we consider to be adequate figures. We hope to obtain earlier ontogenetic stages during the coming summer which will render possible a more extended account. The chief facts observed and conclusions arrived at are as follows:—

1. The lizard is strictly viviparous and not ovo-viviparous, no trace of shell or albumen having been observed.

2. An *omphaloplacenta* is present, which is in a state of regression, but shows some evidence of having been more actively functional at an earlier stage.

3. A true *allantoplacenta* is present, comprising a uterine part of vascular villous ridges with modified epithelium, and a foetal part with greatly modified chorionic ectoderm and a complex allantochorion.

4. The main allantoic blood vessels passing to the placental area are carried across the cavity by means of a remarkable fold in the inner wall.

5. The occurrence of true placentation in two not very closely related Scincid lizards, *Lygosoma* and *Chalcides*, in Australia and Europe respectively, indicates that the allantoplacenta is a functional adaptation, which may have arisen independently many times in evolution, and upon the mere occurrence of which phylogenetic statements cannot justifiably be based.

6. A consideration of reptilian placentation suggests to us three stages of placentation, *chorioplacenta*, *omphaloplacenta*, and *allantoplacenta* arising in that serial order both in ontogeny and in phylogeny.

References.

Bles, 1905.—The Life-history of *Xenopus laevis* Daud. *Trans. Roy. Soc. Edin.*, xli, pp. 789-821.

FLYNN, 1922.—The Phylogenetic Significance of the Marsupial Allantoplacenta. PROC. LINN. SOC. N.S.W., xlvii, 4, pp. 541-544.

————, 1923.—On the Occurrence of a true Allantoplacenta of the conjoint type in an Australian Lizard. *Rec. Aust. Mus.*, xiv, pp. 72-77.

GIACOMINI, 1891.—Matériaux pour l'étude du développement du *Seps chalcides*. *Arch. Ital. de Biol.*, xvi, pp. 332-359. (Reprint from *Monitore Zool. Ital.*, Ann. ii, Nos. 9-10.)

————, 1891*a*.—Über die Entwickelung von *Seps chalcides*. *Anat. Anz.*, vi, pp. 548-551.

————, 1893.—Contribution à la connaissance des annexes fœtales chez les Reptiles, 1e et 2e. Note préventive. *Arch. Ital. de Biol.*, xviii, pp. 336-349. (Abstract of paper in *Monitore Zool. Ital.*, iii, Nos. 6-9.)

————, 1894.—Sur l'oviducte des Sauropsides. *Arch. Ital. de Biol.*, xxi, pp. 147-151. (Abstract of paper in *Monitore Zool. Ital.*, iv, Nos. 10-12, 1893.)

————, 1894*a*.—Nouvelle contribution à la connaissance plus parfaite des annexes fœtales chez les reptiles; Réception du sac vitellin et de l'allantoïde dans la cavité abdominale. *Arch. Ital. de Biol.*, xxi, pp. 151-154. (Abstract of paper in *Monitore Zool. Ital.*, iv, No. 7, 1893.)

HAACKE, 1885.—Ueber ein neue Art uteriner Brutflege bei Reptilien. *Zool. Anz.*, viii, pp. 435-439.

HILL, 1897.—The Placentation of *Perameles*. *Quart. Journ. Micr. Sci.*, xl, pp. 385-442.

————, 1910.—The Early Development of the Marsupialia, etc. *Quart. Journ. Micr. Sci.*, lvi, 1, pp. 1-134.

KERR, 1919.—Text-book of Embryology: II, Vertebrata. London, Macmillan.

LUCAS and FROST, 1893.—The Lizards indigenous to Victoria. *Proc. Roy. Soc. Vict.*, 1893, pp. 24-92.

LUCAS and LE SOUEF, 1909.—The Animals of Australia. Melbourne, Lothian.

SCHAUINSLAND, 1906.—Die Entwickelung der Eihäute der Reptilien und der Vögel, in Hertwig's Handbuch, i, 2, pp. 177-234.

STRAHL, 1891.—Placenta und Eihäute, in Merkel u. Bonnet, *Ergebnisse der Anat. u. Entwick.*, i, pp. 557-8.

STUDIATI, 1851.—Intorno alle connessione dell'uovo coll'ovidutto nel *Seps tridactylus*. *Mem. Accad. Torino*, 2, xv, pp. 101-113.

EXPLANATION OF PLATES XLVII-XLIX.

Lettering.

All. allantois; *All. Ch.* allantochorion; *All. St.* allantoic stalk; *Amn.* amnion; *Amn. C.* amniotic cavity; *Cap.* capillary; *C.b.v.* chorionic blood-vessel; *C.E.* chorionic ectoderm; *C.T.* connective tissue; *Ch.* chorion; *Co.* coagulum; *Foet. Plac.* foetal placenta; *I.A.W.* inner allantoic wall; *Mus.* muscle; *Plac.* placenta; *Pl.* pleated fold of inner allantoic wall; *S.A.C.* sero-amniotic connection; *U.A.* (1 & 2) umbilical arteries; *U. St.* umbilical stalk; *U.T.* uterus; *U.V.* umbilical vein; *Ut. W.* uterine wall; *Ut. b. v.* main uterine blood-vessels; *Ut. Cap.* uterine capillaries; *Ut. plac.* uterine placenta; *Ut. V.* uterine villous ridge; *Vac.* vacuole; *V.A.* (1 & 2) vitelline arteries; *V.V.* vitelline vein; *Y.G.* yolk-granules; *Y.S.* yolk-sac; *Y.S. Plac.* yolk-sac placenta; *Y. St.* yolk-stalk.

Plate xlvii.

Fig. 1.—Left uterus of *L. entrecasteauxi* from dorsal aspect with three embryos, showing placental areas.

Fig. 2.—Foetus of same with uterine wall and foetal membranes partially dissected away to show general relations.

Fig. 3.—Stereogram to show relations of allantois and yolk-sac, with their respective stalks and placental regions.

Plate xlviii.

Fig. 1.—Photomicrograph of total preparation of uterine placental area, showing villous ridges and uterine blood-vessels.

Fig. 2.—Photomicrograph of section of the allantoplacental region showing general relations of foetal and maternal membranes.

Fig. 3.—Photomicrograph of section of uterine wall showing villous ridges and their capillary vessels.

Fig. 4.—Photomicrograph of section of portion of embryo showing reduced yolk-sac passing round head region, with coagulum lying between serosa and uterine wall.

Fig. 5.—Photomicrograph showing yolk-sac at a somewhat earlier stage, with contained coagulum.

Plate xlix.

Fig. 1.—Section of portion of foetal allantoplacenta showing transition of chorionic ectoderm from placental to extra-placental condition.

Fig. 2.—Section of allantoplacental region showing apposition of chorionic ectoderm to maternal villous ridges.

Fig. 3.—Section of omphaloplacental region showing details of foetal membranes and uterine wall.

Fig. 4.—Section of portion of yolk-sac wall.

Fig. 5.—Section of margin of allantoplacental region showing method of transference of allantoic blood-vessels across cavity.

Fig. 6.—Section of umbilical stalk.

Fig. 7.—Section through 4 mm. embryo of *Tiliqua scincoides* to show early thickening of chorionic ectoderm over embryonic area.